

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61L 2/02	A1	(11) International Publication Number: WO 00/43048 (43) International Publication Date: 27 July 2000 (27.07.00)
(21) International Application Number: PCT/GB00/00123 (22) International Filing Date: 19 January 2000 (19.01.00) (30) Priority Data: 9901139.7 19 January 1999 (19.01.99) GB 9910476.2 7 May 1999 (07.05.99) GB (71) Applicant (for all designated States except US): COMMON SERVICES AGENCY [GB/GB]; Trinity Park House, South Trinity Road, Edinburgh EH5 3SE (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): FOSTER, Peter, Reynolds [GB/GB]; Flat 3F1, 5 St. Stephen Street, Edinburgh EH3 5AN (GB). WELCH, Anne, Gillian [GB/GB]; 31 The Firs, Dalgety Bay, Fife KY11 9UH (GB). (74) Agents: MCCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: TREATING PROTEIN-CONTAINING LIQUIDS (57) Abstract Abnormal infective prion proteins are removed from liquids containing biologically active substances, particularly blood products, by filtration using a depth filter formed of a matrix comprising solid particles of porous material and having a pore size less than 6 microns. Preferably the filter is free of cationic or anionic charged material.		

TREATING PROTEIN-CONTAINING LIQUIDS

The present invention relates to a method for the removal of abnormal infective prion proteins associated with transmissible spongiform encephalopathies (TSEs) from an aqueous liquid containing natural products, especially biologically active proteins. The invention also relates to proteins (including foodstuffs and biologically active proteins) and medicinal compositions therefrom where the infective prion has been removed or inactivated.

There is concern about the potential transmission of TSEs such as Creutzfeldt-Jakob Disease (CJD) via whole blood or blood derived biopharmaceuticals. This concern has been heightened by a postulated link between bovine spongiform encephalopathy (BSE) and a new variant form of CJD (vCJD) in humans. CJD is a progressive neurodegenerative disease caused by an unusual infectious agent that replicates in the lymphoreticular tissue and the central nervous system of its host. The nature of the agent is unresolved at present but two main hypotheses have been advanced. The first is the prion or infectious protein hypothesis; and the second is the virion hypothesis which encompasses a combination of host encoded protein with regulatory nucleic acid.

Medicinal compositions for intravenous infusion, intramuscular infusion and topical application have been prepared from human blood plasma for over four decades in a specialised but significant section of the pharmaceutical processing industry. A principal area of concern in the

safety of these products has been potential contamination with blood born viruses. However, the development of screening methods together with technology for the inactivation or removal of potentially contaminating viruses has greatly improved the safety of blood and preparations derived therefrom.

There is currently considerable concern about the possibility that biopharmaceutical products from human or animal sources may transmit TSEs. Although the precise nature of the infective agent in TSEs is at present unclear, TSEs such as Scrapie in sheep and CJD or vCJD in humans are associated with abnormal prion related proteins (PrPs). Suitable screening methods have not yet been developed for abnormal PrPs, which are also extremely resistant to physical and chemical means of inactivation. For example, the EEC regulatory document (CPM Guidelines for Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathies by Medicinal Products: Biologicals, 20, pp155-158, 1992) recommends autoclaving at about 130°C for up to an hour, treatment with 1N sodium hydroxide for 1 hour or treatment with sodium hypochlorite for 1 hour. Such techniques are, however, quite unsuitable for the treatment of biologically active protein - containing materials since they result in total inactivation of the protein.

There is therefore a need to develop methods of removal or inactivation of abnormal infective prion proteins from animal, or human derived medicinal or food products which

are effective yet do not substantially degrade and/or remove the biological activity or food value of the product.

A major problem relates to the ill defined nature of the abnormal prion protein. The normal form of this protein is found in mammalian cells and is present in high levels in brain and lymphoreticular tissues. It is composed of highly membrane associated 33-35KDa phosphoinositol glycoprotein, which is completely sensitive to digestion with proteinase K. The infectious (abnormal) form of the protein has been shown to exist in an altered conformational form, contains a high level of β pleated-sheet, and is resistant to digestion with proteinase K. The change in conformation is thought to result in the protein becoming highly insoluble, forming aggregates which then deposit in the infected tissue as fibrils or amyloid plaques. The unknown properties of the abnormal prion proteins, and particularly the state of aggregation makes the prediction of suitable removal or inactivation techniques very difficult.

In the prior art, removal or inactivation by chromatographic techniques has been attempted. Hunter and Millson (J. Gen. Microbiol., 1964, vol. 37 pp251-258) showed examples of the chromatographic behaviour of scrapie-infected brain homogenate on DEAE cellulose adsorption and calcium phosphate columns. International patent application W097/3454 relates to the removal of abnormal prion proteins from solutions of albumin or

reagent grade animal serum using typically expensive mixed ion exchange and hydrophobic solid phases.

Blum et al (BioPharm. 11(4) pp28-34, 1998) investigated the effectiveness of various steps (i.e. heating, precipitation, absorption with filter aid and ion-exchange chromatography) in the production of aprotinin and bovine serum albumin in removing added spikes of scrapie as a model source of TSE. However, it is unclear which of the above elements are essential or required in the removal of the scrapie agent.

Patent specification EP0798003 discloses filtration as a way of removing unwanted contaminants. A positively charged depth filter of 0.25 to 2.0 micron pore size also carrying a cation resin, was used for the removal of viruses from biologically active protein solutions. Morgenthaler (TSE issues, Cambridge Health Tech. Institute CHI, November, 1998, Lisbon, Portugal) has shown that filtration steps (including nanofiltration) can substantially remove added TSE spikes in the fractionation of human plasma.

It is an object of the present invention to further develop and characterise the removal of abnormal infective prion proteins from protein-containing liquids, particularly those derived from human plasma, without unacceptable effects on the nature or biological activity of the proteins.

It is a further object of the present invention to provide a depth filter which can be a single use filter and

may be disposed of having removed the abnormal prion proteins from the process stream.

The invention is based on the surprising discovery that filtration using a depth filter comprising particles and having a pore size less than six microns is surprisingly effective in removing abnormal infective prion proteins.

In particular, the invention provides a method for the removal of abnormal infective prion proteins associated with transmissible spongiform encephalopies (TSEs) from an aqueous liquid containing a natural product (especially a biologically active protein), which comprises passing the liquid through a depth filter formed of a matrix comprising solid particles of porous material and having a pore size providing a retention less than $6\mu\text{m}$. Typically the filter may be a single use disposable filter.

By the term "removal" is meant the actual physical removal of the abnormal infective prion protein from the liquid containing the desired protein. For practical purposes, the recovery of the desired protein in its original biological state should be substantially maintained at least to a level in excess of 50%, preferably 80%, more preferably 90%.

Removal of the abnormal infective prion protein may be achieved to an extent of at least $10^{2.5}$, 10^3 , preferably 10^4 , more particularly 10^5 .

The pore size of the filter matrix is preferably in the range 0.6 to 6 microns, particularly 0.6 to 1.5

microns. The pore size is defined in terms of the particle size of particles retained thereon. Typically particles of defined size such as micro-organisms are used for calibration purposes.

The invention also relates to the treated liquid.

Of particular importance to the fractionation of blood plasma products, is the discovery that filtration may be effectively carried out under non-denaturing conditions for the biologically active protein, and under conditions which do not reduce the solubility of the product protein. In addition filtration with or without filter aid can be used to remove suspended solids.

The method may be carried out at a pH in the range 4-10, preferably 5-9, and especially 6-8.

The application of heat is unnecessary and the process can be conducted at substantially room temperature or below, in particular in the range -5 to +20°C.

Preferably, the liquid and the filter are free of cationic or anionic charged material which may contribute to the reduction of biological activity of the biologically active protein, and in particular may cause activation of sensitive blood coagulation factors. The process is in particular applicable to the treatment of whole blood or liquids containing albumin, immunoglobulins, Factor IX, thrombin, fibronectin, fibrinogen, Factor VIII and Factor II, VII, IX and X and other proteins derived from plasma. It is also applicable to the treatment of plasma, Factor XI, Factor XIII, haemoglobin, alpha-2-macroglobulin,

haptoglobin, transferrin, apolipoprotein, mannan binding protein, protein C, protein S, caeruloplasmin, C-1-esterase inhibitor, inter-alpha-trypsin inhibitor, Van Willebrand factor. Recombinant analogues of these may also be treated. In addition, the invention is applicable to the treatment of other natural products including foods, drinks, cosmetics etc. It is also applicable to other non-plasma animal-derived products, such as heparin and hormones.

The depth filter generally comprises a binder, such as cellulose, together with a solid porous particulate material such as Kieselguhr, perlite or diatomaceous earth.

The depth filter generally has a thickness in the region 1-10mm, particularly 2-5mm. The material used for the depth filter should have little or no effect on the desirable protein concerned.

Embodiments of the present invention will now be described by way of example only.

METHODOLOGY

1) Preparation of hamster scrapie spike

Hamster adapted scrapie (H_s) agent (strain 263K) was prepared by homogenisation of infected brain tissue in phosphate buffered saline. The titre of the agent produced in this way is normally of the order of 10^7 - 10^9 LD₅₀ units ml⁻¹ as assayed by the intracranial route in hamster. A stock of the hamster adapted scrapie strain agent (263K) is stored at or below -70°C.

A microsomal fraction derived from crude brain homogenate was used for all spiking experiments.

The microsomal fraction was prepared according to the method of Millson et al (Millson GC, Hunter GD and Kimberlin RH (1971); "An experimental examination of the scrapie agent in the cell membrane mixtures. The association of scrapie activity with membrane fractions", J. Comp Path. 81, 255-265). Crude brain homogenate prepared from 263K infected brains by Dounce homogenisation was pelleted at 10,000g for 7min to remove nuclei, unbroken cells and mitochondria. The microsomes remaining in the supernatant were then pelleted by centrifugation at 100,000g for 90min, followed by resuspension in PBS.

2) Calculation of results

Clearance (C) and reduction (R) factors were calculated based on the end point dilution for samples after analysis by Western blotting. The end point dilution is calculated based on the first dilution at which no scrapie prion protein (PrP^{sc}) can be detected. The reciprocal of this dilution is then taken as the titre of agent, and thus all titres are expressed in arbitrary units. Based on the titre determined by end point dilution, the total amount of PrP^{sc} in the sample is calculated based on the volume of the sample and taking into account any correction factors which need to be applied. Clearance factors are calculated relative to the theoretical input spike. Reduction factors are calculated

relative to the level of PrP^{sc} detected in the load sample.

Where no PrP^{sc} is detected at the highest concentration of sample tested, then the reciprocal of the dilution is taken as 1, and clearance and reduction factors are expressed with a \geq sign proceeding the logarithmic value.

3) Western Blot Assay for Scrapie Infectivity

The titre of the stock of 263K used in this study, as well as the titre present in all samples generated during the study was determined by a Western blot procedure. This procedure relies upon the difference in susceptibility of the infectious (PrP^{sc}) and non-infectious (PrP^c) to proteinase K digestion. Samples were treated with protease K to digest away any PrP^c, and run on a SDS polyacrylamide gel followed by blotting onto nitrocellulose. Any PrP remaining after protease digestion, corresponding to PrP^{sc}, was then detected using a PrP specific antibody. The relative level of scrapie in the samples compared to the stock was determined by serial dilution to end point (the point where no signal was detected) of all samples.

Further information is given in A. Bailey, "Strategies for the Validation of Biopharmaceutical Processes for the Removal of TSE's", Cambridge Healthtech Institute, Nov.1998, Lisbon, Portugal.

Table 1 shows the efficiency of removal of spiked hamster scrapie prion proteins (PrP^{sc}) by various depth filters. Removal is expressed as clearance factor C (amount from inoculum/amount in filtrate) or as reduction

factor R (amount in feedstock/amount in filtrate). The Seitz KS80 filter of pore size 0.6 to 1.5 microns according to the present invention is highly effective in removing the prion proteins. Other filters presented for comparison purposes having either a larger pore size or including cationic species are less effective.

EXAMPLES

Example 1 (Treatment of Albumin according to the invention)

A model system was set up to replicate on an experimental scale the depth filtration of albumin in the conventional plasma fractionation process, employing different types of filter. The albumin-containing sample (fraction V) was spiked with hamster scrapie prion protein produced as described above and the concentration thereof was assessed by Western Blotting also as described herein.

The filter was a Seitz KS80 (trademark) pad cut to a 142mm diameter disk of effective filtration area 128cm². The filter was pretreated by passing ethanol and NaCl through for 35 minutes. The sample material was approximately 1 litre of resuspended fraction V at pH6.9 and 85.0g/l concentration taken from the conventional plasma fractionation process and kept at +4°C.

The conventional process involves the batch filtration of 853ml of sample. In this experiment, the same total volume of sample was passed through the filter, but only the final 100ml was spiked with microsomal hamster scrapie. 100ml of the sample starting material was spiked with 9.5ml

of the preparation of microsomal hamster (263k) scrapie and a sample of the spiked material was removed for analysis of the level of PrP^{sc}. The spiked material was passed through the filter at a flow rate of 6.4ml/min and the filtrate collected for analysis of the level of PrP^{sc}. All samples were stored at or below -70°C until analysis of the level of PrP^{sc} was carried out. Before the samples were analysed by Western Blotting, any scrapie in the sample was concentrated by ultracentrifugation.

Table 1 shows that the removal assessed by clearance factor C and reduction factor R exceeds four log units (the detection limit) with no abnormal prion protein being detected in the filtrate. The filter is therefore most effective in removing the added spike of hamster scrapie prion protein.

Example 2 (Albumin Treatment - Comparison)

The fraction V albumin-containing sample was filtered with a different filter in a similar manner to Example 1.

The filter used was a Cuno (trademark) Delipid Del 1 mini cartridge of effective filtration area 27cm². The filter was pretreated with ethanol and NaCl.

The sample material was fraction V at pH 6.9 and 85g/l concentration taken after conventional filtration through a Seitz KS80 filter and held at +4°C.

In this case, only the final 50ml were spiked with microsomal hamster scrapie. The spiked material was passed through the filter at a flow rate of 3.2ml/min and the

filtrate analysed for prion protein as before. The extent of clearance is shown in Table 1. Clearance and removal were only 2.8 and 2.3 logs respectively; abnormal prion protein being detected in the filtrate.

Example 3 (IGG - Comparison)

The procedure of Example 1 was repeated on IgG-containing supernatant I and III from conventional plasma fractionation.

The filter was a Millipore lifegard CP20 disk 47mm in diameter providing an effective filtration area of 12.5cm². The sample material was about 800ml of supernatant I and III (prefiltration) from the conventional plasma fractionation procedure and held at +4°C. It contained about 12% ethanol and had a pH 5.1.

680ml of sample was treated and the final 50ml was spiked with microsomal hamster scrapie.

The extent of removal of prion proteins is given in Table 1. The clearance factor C was 3.0 logs and the removal R was less than 1 log; abnormal prion protein being detected in the filtrate.

Example 4 (IGG - Invention)

The general procedure of Example 1 was repeated on IgG-containing fraction II suspension from conventional plasma fractionation.

The filter was a Seitz K200 (trademark) of 142mm diameter and effective filtration area 128cm². The sample

material was resuspended fraction II from conventional plasma fractionation held at +4°C.

600ml of sample was passed through the filter and the final 100ml was spiked with microsomal hamster scrapie. The extent of removal of the PrP^{sc} was determined as before and the results are given in Table 1. The clearance C and removal R both showed no abnormal prion protein in the filtrate to the limit of detection ($C \geq 3.4$ and $R \geq 2.8$).

Thus, it can be seen from the Examples that greater than 10^4 times removal of abnormal infective prion proteins can be achieved using a neutral filter having a pore size of less than 2 microns, and greater than $10^{2.5}$ times removal using a neutral filter of pore size 3.5 to 6.0 microns. These are the limits of detection. In other words no detectable abnormal prion protein was present in the filtrate.

Example 5 - (fibre filters-Comparison)

Four filters formed of fibres were tested for prion removal in a manner similar to Example 1.

Whole blood was spiked with 3.8 \log_{10} of hamster scrapie (Hs 263K) and the clearance C and removal R determined.

The results given in Table 2 shows that these fibre filters gave < 1 log removal and were therefore ineffective for removing prion proteins.

TABLE 1**REMOVAL OF PrP^{sc} BY DEPTH FILTRATION**

FILTER (PRODUCT)	COMPOSITION	RETENTION (μm)	C	R
Seitz, KS80 (Albumin)	Cellulose, Kieselguhr Perlite	0.6 - 1.5	≥ 4.1	≥ 4.9
Seitz, K200P (IgG)	Cellulose, Kieselguhr Perlite	3.5 - 6.0	≥ 3.4	≥ 2.8
Cuno, Delipid 1 (Albumin)	Cellulose, Kieselguhr Cation Resin	0.6	2.8	2.3
Millipore CP20 (IgG)	Borosilicate glass	2.0	3.0	<1 *

* Large drop in PrP^{sc} measured after addition of inoculum to process feedstock.

TABLE 2

FILTER (PRODUCT)	COMPOSITION	RETENTION (μm)	C	R
Baxter/Asahi Optiplus R7456(RS2000)	Negatively charged polyester fibres	n/a	< 1	< 1
Fresenius whole blood filter	Melt blown woven-non- woven polyester fibre	n/a	< 1	< 1
compoflex T2916	Non-ionic surface			
Macopharma eucoflex LST-1	Nonwoven non ionic surface polyester/ polypropylene mixture 4 layers polyester/22 layers polypropylene/ 1 layer polyester	6-13	< 1	< 1
Pall leukotrap BPF-4	Non-woven polyester negatively charged	n/a	< 1	< 1

n/a - not available

CLAIMS

1. A method of removal of abnormal infective prion proteins associated with transmissible spongiform encephalopies (TSEs) from an aqueous liquid containing a natural product, which comprises passing the liquid through a depth filter formed of a matrix comprising solid particles of porous material and having a pore size providing a retention less than 6 μ m, and so removing any abnormal infective prion proteins which may be present in the liquid.
2. A method according to claim 1 wherein the matrix comprises a binder.
3. A method according to claim 2 wherein the binder is cellulose.
4. A method according to any preceding claim wherein the solid porous particles are kieselguhr or perlite particles or mixtures thereof.
5. A method according to any of claims 1 to 3 wherein the solid porous particles are diatomaceous earth particles.
6. A method according to claim 1 carried out in the absence of cationic or anionic charged material.

8. A method according to claim 1 wherein the pore size is in the range 0.6 to 6 microns.

9. A method according to claim 1 wherein the pore size is in the range 0.6 to 1.5 microns.

10. A method according to claim 1 wherein the depth filter has a thickness of 2 to 5 mm.

11. A method according to claim 1 wherein the natural product is a protein.

12. A method according to claim 1 wherein the aqueous liquid comprises a blood plasma product.

13. A method according to claim 12 wherein the blood plasma product albumin, an immunoglobulin, Factor IX, thrombin, fibronectin, fibrinogen, Factor VIII, Factor II, Factor VII, Factor IX, or Factor X.

INTERNATIONAL SEARCH REPORT

National Application No

PCT/GB 00/00123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L2/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	FOSTER P R: "Assessment of the potential of plasma fractionation processes to remove causative agents of transmissible spongiform encephalopathy." TRANSFUSION MEDICINE, (1999 MAR) 9 (1) 3-14. REF: 60 , XP000904838 the whole document	1-14
X	GB 2 045 828 A (AMF INC) 5 November 1980 (1980-11-05) page 3, line 30 - line 40; claims -/-	1-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

17 April 2000

Date of mailing of the international search report

02/05/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/00123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GALE, P. ET AL: "Development of a risk assessment for BSE in the aquatic environment." JOURNAL OF APPLIED MICROBIOLOGY, (APRIL, 1998) VOL. 84, NO. 4, PP. 467-477. , XP000904839 the whole document	1-14
A	US 5 696 236 A (MORGENTHALER JEAN-JACQUES ET AL) 9 December 1997 (1997-12-09) claims; examples 1-8	1-14
A	WO 96 05846 A (SANOELL PHARMA GMBH & CO ;NEBE CARL THOMAS (DE)) 29 February 1996 (1996-02-29) claims	1-14
A	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US SIMON, S.: "Enzymic reactions and reactors containing enzymes crosslinked to a filter containing inorganic materials." retrieved from STN Database accession no. 100:64118 XP002135499 abstract & IL 59 339 A (RESEARCH PRODUCTS REHOVOT LTD., ISRAEL) 30 September 1982 (1982-09-30)	1-14
A	HOU K ET AL: "CAPTURE OF LATEX BEADS, BACTERIA, ENDOTOXIN, AND VIRUSES BY CHARGE-MODIFIED FILTERS" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, US, WASHINGTON, DC, vol. 40, no. 5, 1 November 1980 (1980-11-01), pages 892-896, XP002057339 ISSN: 0099-2240 abstract	1-14
A	DE 29 10 289 A (AMF INC) 25 September 1980 (1980-09-25) claims	1
A	EP 0 798 003 A (BAYER AG) 1 October 1997 (1997-10-01) cited in the application examples 1,2	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/00123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2045828 A	05-11-1980	AU 5655680 A	09-10-1980
		BR 8002082 A	23-12-1980
		CA 1148872 A	28-06-1983
		DE 3010581 A	16-10-1980
		FR 2452966 A	31-10-1980
		JP 1268677 C	10-06-1985
		JP 55134620 A	20-10-1980
		JP 59041765 B	09-10-1984
		US 4305782 A	15-12-1981
		US 4366068 A	28-12-1982
US 5696236 A	09-12-1997	EP 0679405 A	02-11-1995
		AU 689546 B	02-04-1998
		AU 1764295 A	02-11-1995
		CA 2147797 A	26-10-1995
		JP 2987554 B	06-12-1999
		JP 8040920 A	13-02-1996
		NZ 270987 A	27-08-1996
WO 9605846 A	29-02-1996	DE 4429558 A	22-02-1996
		AT 180672 T	15-06-1999
		AU 3252095 A	14-03-1996
		DE 59506111 D	08-07-1999
		EP 0776212 A	04-06-1997
		ES 2135079 T	16-10-1999
		GR 3030688 T	30-11-1999
IL 59339 A	30-09-1982	NONE	
DE 2910289 A	25-09-1980	GB 2043734 A	08-10-1980
		FR 2452310 A	24-10-1980
		GB 2098590 A, B	24-11-1982
		JP 1478924 C	10-02-1989
		JP 55129124 A	06-10-1980
		JP 63017486 B	14-04-1988
EP 0798003 A	01-10-1997	AU 1636097 A	25-09-1997
		CA 2200148 A	21-09-1997
		JP 10028581 A	03-02-1998